

USER MANUAL

CytoQuest™ CR

Non-invasive, Circulating Rare Cell Isolation and Retrieval System



Content

Chapter 1	Safety Information	2
Chapter 2	System Overview	4
2.1	<i>Background</i>	4
2.2	<i>Description</i>	5
2.3	<i>Consumable Replacement Schedule</i>	8
2.4	<i>Workflow</i>	16
Chapter 3	Required Materials	17
Chapter 4	Reagent Preparation	19
Chapter 5	Blood Collection and Handling	21
5.1	<i>Phlebotomy</i>	21
5.2	<i>Transportation</i>	21
Chapter 6	Cell Enrichment	22
6.1	<i>Leucosep Preparation</i>	22
Chapter 7	Capture Antibody Immobilization	24
Chapter 8	Cell Capture	25
8.1	<i>Parameters and Settings</i>	25
8.2	<i>System Priming</i>	28
8.3	<i>Buffer Filling</i>	29
8.4	<i>Sample Loading and Capture</i>	33
8.5	<i>Cell Fixation</i>	36
8.6	<i>Tubing Cleaning</i>	37
Chapter 9	Cell Immunostaining	38

Chapter 10	Cell FISH	40
Chapter 11	System Maintenance	43
	<i>11.1 General Maintenance</i>	<i>43</i>
	<i>11.2 System Cleaning</i>	<i>44</i>
Chapter 12	System Specifications	45

Safety Information

Users should always be aware of the following general safety precautions when operating this instrument. Using this instrument in a manner not specified in this manual may result in personal injury or damage to the instrument. Abnova (Taiwan) Corp. assumes no liability for the customer's failure to comply with these precautions.

Safety Notices

1. To prevent electric shock, users should keep hands, equipment, clothing, work areas dry at all times.
2. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
3. The instrument should only be installed in the environment with good ventilation.
4. The system must be installed and maintained by an Abnova (Taiwan) Corp. Technical Representative.
5. Wear appropriate personal protective equipment during operation (for example, safety goggles, gloves, or protective clothing).
6. Ensure that anyone who operates the instrument has received instructions in both general safety training for laboratories and specific safety training for the instrument.

7. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves.
8. Do not attempt to reuse the consumable parts and materials.

System Overview

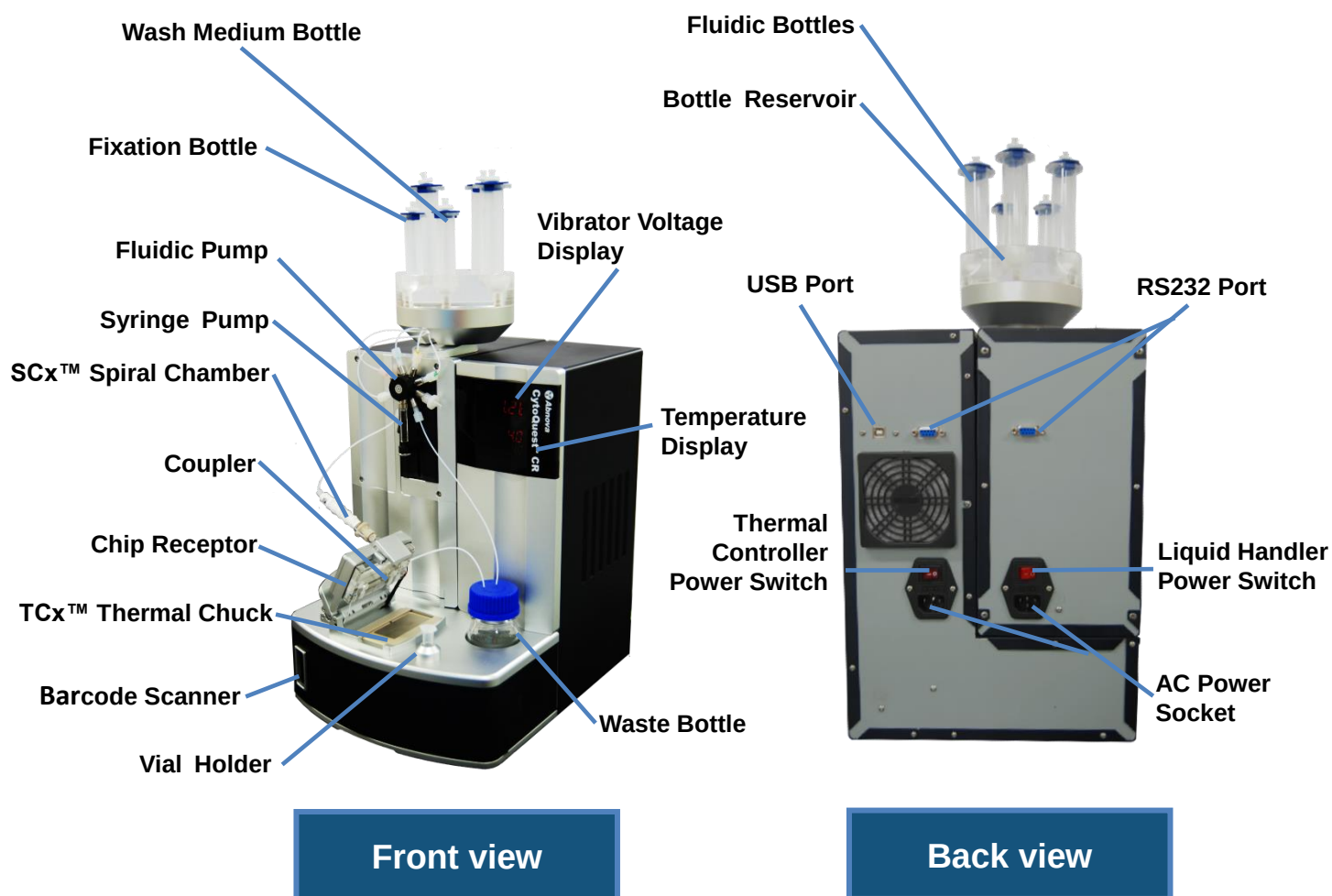
2.1 Background

Abnova's CytoQuest™ CR is a non-invasive system for capture, enumeration, isolation and retrieval of circulating rare cells (CRCs). Three major subtypes of CRCs in translational research and clinical studies are circulating tumor cells (CTCs), circulating stem cells (CSCs), and circulating fetal cells (CFCs). A challenge for market adoption of CRCs is the efficient and reproducible identification, single cell isolation, and retrieval of highly pure and viable CRCs, with their applications supported by a wide repertoire of standardized, GMP grade bioreagents.

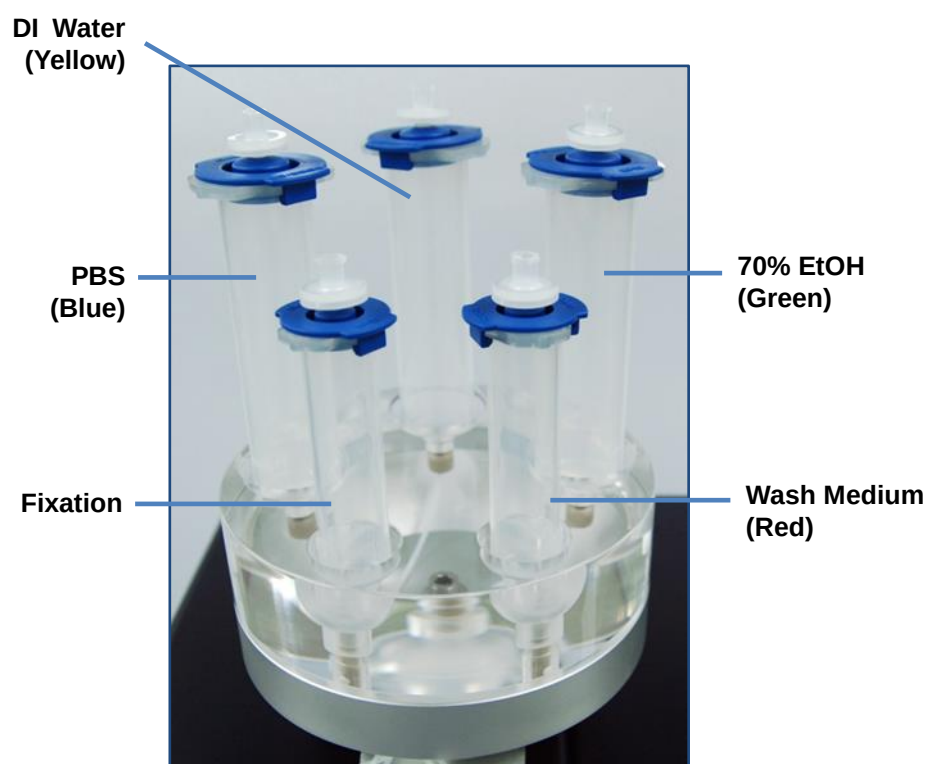
CytoQuest™ CR technology utilizes SCx™ spiral chamber, HBx™ micromixer, antibody immobilized nanoarray, and TCx™ thermal control to enable a multitude of CRC functions. SCx™ spiral chamber is equipped with a non-sticky coil and a self-contained Microvibrator for unimpeded delivery of pretreated blood sample into the nanoarray. HBx™ micromixer provides a herringbone conduit for cell mixing. CytoChipNano is a streptavidin nanoarray which captures the CRCs for cell enumeration and single cell isolation by laser microdissection or micromanipulation. CytoChipNano CR is a specialized streptavidin nanoarray with thermo-sensitive coating which captures and releases the CRCs via a TCx™ thermal control of alternating temperatures.

Monoclonal antibodies targeting specific surface biomarkers of circulating rare cells can be flexibly interchanged to accommodate the biomarker switch in CRC progression. This results in highly pure and viable CRCs for downstream protein characterizations, gene analyses, and cell assays. Cell enumeration is currently the only FDA approved application as a prognostic marker in breast, prostate, and colorectal cancers. In contrast, effective single cell isolation and retrieval of CRCs opens up new scope of applications in the diagnostic and pharmaceutical industry.

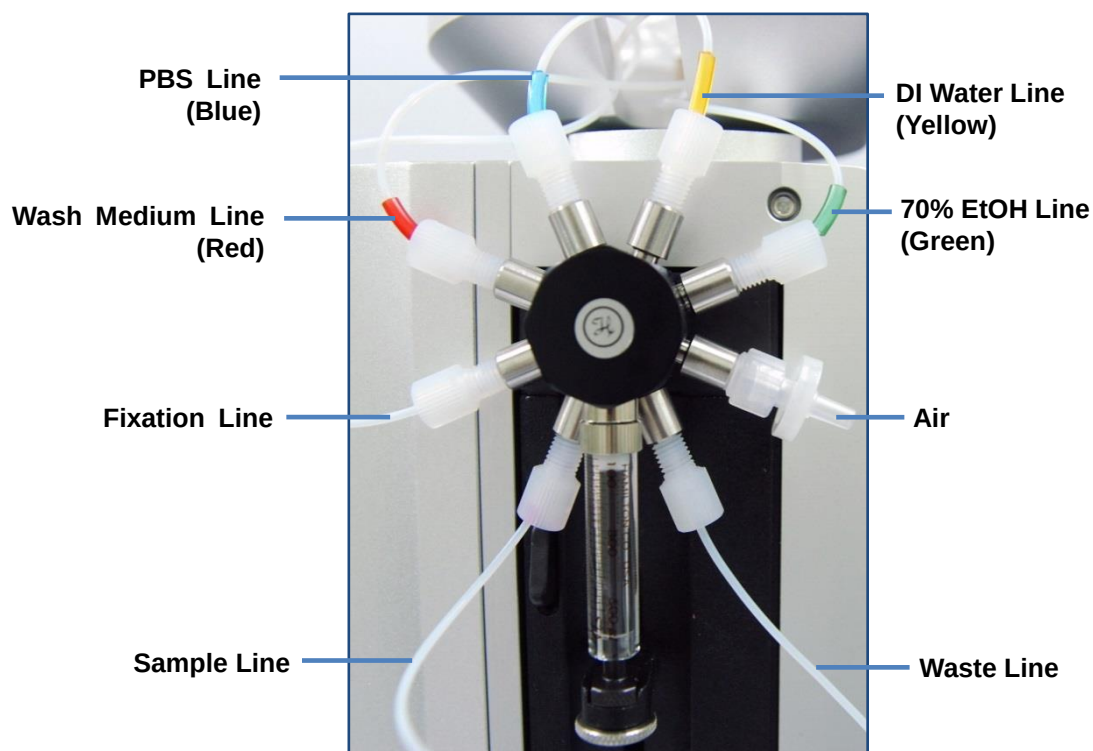
2.2 Description



- **CytoQuest™ CR system, SCx™ spiral chamber, HBx™ micromixer and TCx™ thermal control are trademarks and pending patents of Abnova (Taiwan) Corporation.**



Bottle View



Fluidic view

2.3 Consumable Replacement Schedule

To avoid cross contamination, user should replace CytoChipNano, Coil Holder, Connecting base of SCx™ Spiral Chamber, Inlet Plug and Outlet Plug when applying new sample in Cell Capture. See Consumable Replacement Schedule below.

Items	Sub-Items	Usage	Replacement Schedule	Abnova Catalog#
Fluidic Bottles	70% EtOH Bottle	Reusable	System notice	KA4442
	DI Water Bottle	Reusable	System notice	
	PBS Bottle	Reusable	System notice	
	Wash Medium Bottle	Reusable	System notice	
	Fixation Bottle	Reusable	System notice	
Fluidic Tubings	70% EtOH Line (Green)	Reusable	System notice	
	DI Water Line (Yellow)	Reusable	System notice	
	PBS Line (Blue)	Reusable	System notice	
	Wash Medium Line (Red)	Reusable	System notice	
	Fixation Line	Reusable	System notice	
	Sample Line	Reusable	System notice	
	Waste Line	Reusable	System notice	
	Air (filter)	Reusable	System notice	
Chips	CytoChipNano	Do not reuse*	Every use	U0095
	CytoChipNano CR	Do not reuse	Every use	
	Clean Chip	Reusable*	Every 20 uses	
	Dummy Chip	Reusable*	Every 20 uses	
SCx™ Spiral Chamber	Coil Holder	Do not reuse*	Every use	U0314
	Connecting Base	Do not reuse*	Every use	
	Microvibrator	Reusable	Replace if malfunction	
	Electrode	Reusable	Replace if malfunction	
Plugs	Inlet Plug (Blue)	Do not reuse*	Every use	KA4441
	Outlet Plug (White)	Do not reuse*	Every use	
	Outlet Plug (Blue) with Tubing	Do not reuse*	Every use	
Chip Opener	Razor	Reusable	Replace if malfunction	

*These one-time-use consumables come together with CytoChipNano Combo Pack (CAT# [KA4440](#), Abnova).

2.3.1 Removing Connecting Base from SCx™ Spiral Chamber



(Top to bottom)

Hold the Microvibrator and pull off the Connecting Base.

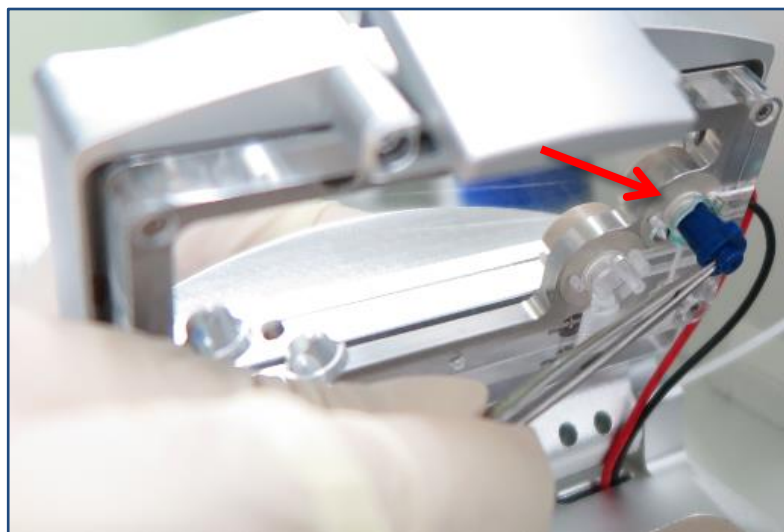
2.3.2 Removing Electorod from Connecting Base



(Top to bottom)

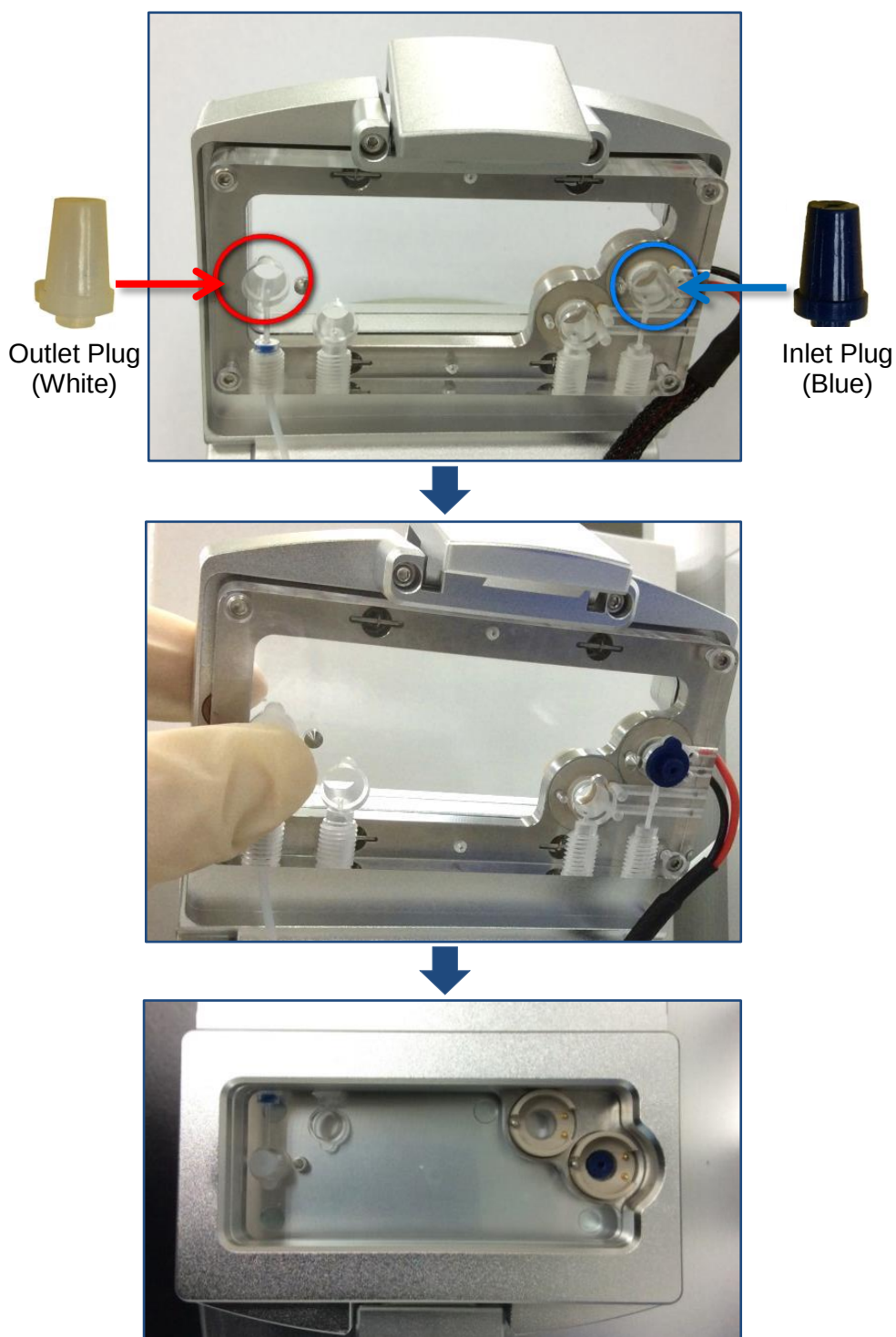
Use finger to remove Electorod from used Connecting Base.

2.3.3 Removing Inlet Plug and Outlet Plug from the Coupler



Insert the tip of the tweezers into the end of plugs at the underside of the Coupler. Loosen the plugs by gently twisting and shaking. Then pull the plugs out with one hand while supporting the Coupler with the opposite hand.

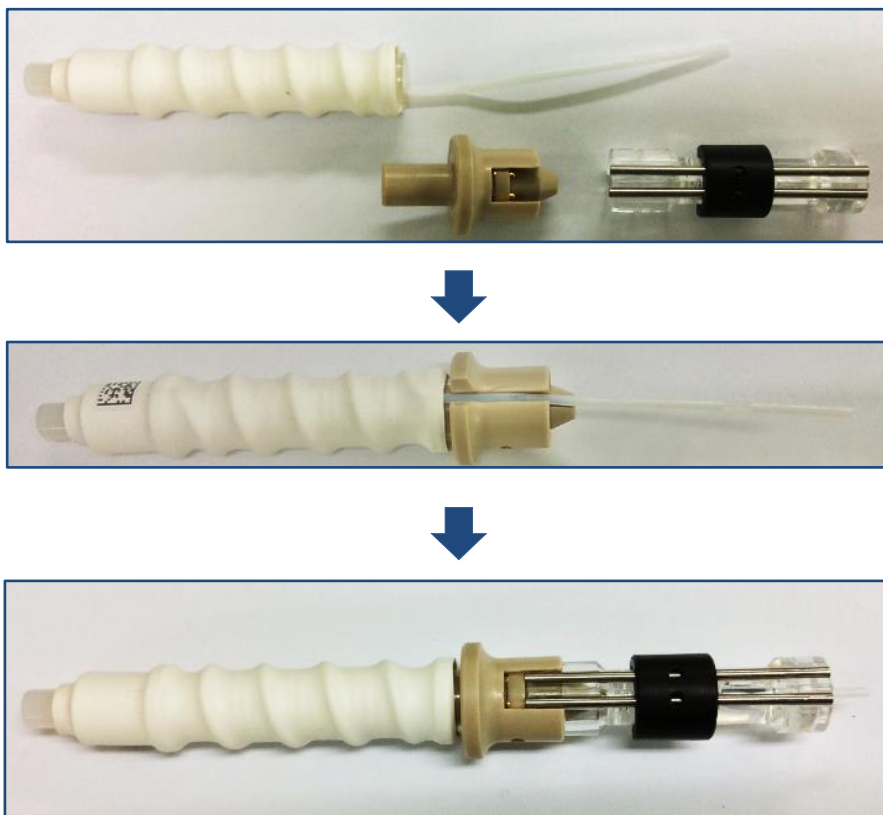
2.3.4 Inserting Inlet Plug and Outlet Plug into the Coupler



(Top to bottom)

1. Open the Coupler and prepare Inlet Plug (blue) and Outlet Plug (white) by cleansing and lubricating the plugs with 70% ethanol first.
2. Align Inlet Plug with the blue circled hole and Outlet Plug with the red circled hole as shown, and then fully insert the plugs from the underside of the Coupler.
3. Close the Coupler gently.

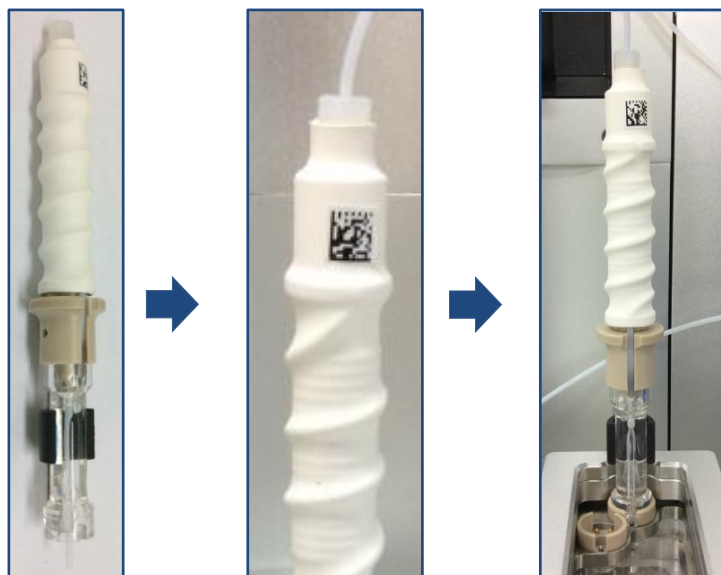
2.3.5 Assembling SCx™ Spiral Chamber



(Top to bottom)

1. Prepare Coil Holder, Microvibrator and Connecting Base of SCx™ Spiral Chamber.
2. Assemble firmly Microvibrator into Coil Holder.
3. Assemble firmly the part from step 2 into Connecting Base while pass the tubing through the center of Connecting Base.

2.3.6 Inserting SCx™ Spiral Chamber into the Chamber Port of the Coupler



(Left to right)

1. Prepare SCx™ Spiral Chamber.
2. Connect Sample Line to SCx™ Spiral Chamber.
3. Insert SCx™ Spiral Chamber into the Chamber Port of the Coupler.

2.4 Workflow

Chapter 5

Blood Collection

Chapter 6.1

Leucosep Preparation
(Operating time: ~30 min)

Chapter 7

**Capture Antibody
Immobilization**
(Operating time: ~60 min)

Chapter 8

CytoQuest™ CR Cell Capture
(Operating time: ~45 min/chip)

Chapter 9

Cell Immunostaining
(Operating time: ~90 min)

Chapter 10

Cell FISH
(Operating time: ~50 hr)

Microscope Examination

 **Operate in a clean laboratory environment.**

 **Operate inside CytoQuest™ CR.**

3

Required Materials

Antibody	Catalog#	Brand
CytoQuest™ Epithelial Cell Kit • Anti-EpCAM capturing antibody (Biotin) • Anti-PanCK detecting antibody (FITC) • Anti-CD45 detecting antibody (PE) • 50X Antibody Dilution Buffer (50X ADB)	KA4439	Abnova

* CytoQuest™ Epithelial Cell Kit is used for Cell Capture and Cell immunostaining described in this manual. User may select other kits for different types of CRCs. Please visit [CytoQuest™ CR](#) for more information.

Reagent	Catalog#	Brand
Bovine Serum Albumin, BSA	BP9706-100	Fishersci
Certified Fetal Bovine Serum, FBS	14-502F	Lonza
CytoQuest™ Epithelial Cell Solution • 50X Antibody Dilution Buffer (50X ADB)	KA3967	Abnova
CytoQuest™ SuperBlocker	U0317	Abnova
DAPI (*For “Cell Immunostaining”)	D1306	Invitrogen
DAPI Counterstain (150ng/ml) (*For “Cell FISH”)	U0030	Abnova
FFPE FISH PreTreatment Kit 1	KA2691	Abnova

Chemical	Catalog#	Brand
20% Paraformaldehyde (formaldehyde) Aqueous Solution	15715-S	Electron Microscopy Science
2-Propanol	109827-1L	Fishersci
Dimethyl Sulfoxide, DMSO	TS-20684	Fishersci
Ethanol	100983	Merck
IGEPAL CA-630 (NP-40)	I-3021	Sigma-Aldrich
Phosphate Buffered Saline, PBS	P-5368	Sigma-Aldrich

Propylene Glycol	215195790	Fishersci
RPMI Medium	12633-012	Invitrogen
Triton X-100	93426-100ML	Sigma-Aldrich

Consumable	Catalog#	Brand
15 mL Centrifuge Tubes	3131-345-008	Labcon
5 mL Dropper	30006-3S1	Alpha Plus
50 mL Centrifuge Tubes	352070	Fishersci
BD Vacutainer™ Plastic Blood Collection Tubes with Sodium Heparin	02-689-6	Fishersci
BD Vacutainer™ Safety-Lok™ Blood Collection Sets with Pre-Attached Holder	02-683-21	Fishersci
Bottle and Tubing Pack	KA4442	Abnova
Coverslip	990	Assistent
Cryovial	02-912-729	Fishersci
CytoChipNano	U0095	Abnova
CytoChipNano Combo Pack • CytoChipNano • SCx™ Spiral Chamber • Plugs	KA4440	Abnova
Eppendorf Micro Test Tubes	0030 121.589	Eppendorf
Mr. Frosty Freezing Container	15-350-50	Fishersci
Histopaque®-1077	10771	Sigma-Aldrich
Leucosep®	163290P	Greiner Bio-One
Plugs	KA4441	Abnova
Rubber Cement	CXA-100	Kokuyo
SCx™ Spiral Chamber	U0314	Abnova

4

Reagent Preparation



All the reagents/solutions used should be of molecular grade and sterile-filtered!

- **1X PBS**

1X PBS consists of 8.1 mM $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 1.46 mM KH_2PO_4 , 138 mM NaCl; 2.7 mM KCl, pH 7.4, at 25°C. 1X PBS without calcium and magnesium can be stored at room temperature.

- **WM (Wash Medium, RPMI with 5% FBS)**

WM is aliquoted and kept frozen at -20°C. Once thawed, WM can be stored at 4°C for up to 3 weeks.

- **1X ADB**

Dilute 50X ADB by adding adequate 1X PBS to 50X ADB. 1X ADB should be kept on ice or at 4°C and used within 2 days.

- **1X ADB/0.2% Triton X-100**

Add 0.2 mL of Triton X-100 to 100 mL of 1X ADB.

- **2% PFA (2% Paraformaldehyde Aqueous Solution)**

Dilute 20% paraformaldehyde aqueous solution with 1X PBS. 2% PFA should be aliquoted and frozen at -20°C. 2% PFA can be stored at 4°C for 1 week.

- **FM (Freezing Medium)**

FM consists of 90% FBS, 5% DMSO, and 5% propylene glycol. FM should be aliquoted and frozen at -20°C. Once thawed, it can be stored at 4°C for up to 3 weeks.

- **2X SSC Wash Buffer**

Dissolve 17.53 g of sodium chloride and 8.82 g of sodium citrate tribasic dehydrate in 700 mL deionized water. Adjust the pH to 7.0 and the volume to 1 L. Following autoclaving sterilization, 2X SSC Wash Buffer can be stored at ambient temperature.

- **2X SSC/0.3% NP-40**

Add 3mL of IGEPAL CA-630 to 990mL of 2X SSC Wash Buffer pre-warmed to 70°C. Bring the final volume to 1 L with 2X SSC Wash Buffer. Store 2X SSC/0.3% NP-40 at ambient temperature.

Blood Collection and Handling

5.1 Phlebotomy

Access to a peripheral vein should be performed with a 23G needle. The first 2 mL of collected blood should be discarded to avoid potential skin cell contamination from venipuncture. Then transfer 10 mL of blood sample in each Heparin tube (green cap). Gently rotate the 10 mL Heparin tube end-over-end five times immediately. Place the collected blood sample at room temperature before use.

5.2 Transportation

Blood has a maximum preservation time of 6 hours at room temperature. For example, if the blood is drawn at 9am in a hospital, it should be processed by 3 pm.

Cell Enrichment

6.1 Leucosep Preparation

- 6.1.1 Warm-up Histopaque®-1077 density gradient media to room temperature (RT).
- 6.1.2 Fill each Leucosep® tube with 3 mL of Histopaque®-1077 density gradient media.
- 6.1.3 Centrifuge the Leucosep® tube at 1000 x g and RT for 30 seconds. The density gradient media should be spun below the porous barrier.
- 6.1.4 Dilute the blood sample at 1:1 ratio with 1X PBS.
- 6.1.5 Carefully pour 4 mL of diluted blood sample into the prepared Leucosep® tube.
- 6.1.6 Centrifuge the Leucosep® tube at 1000 x g and RT for 10 minutes in a swinging bucket rotor.
- 6.1.7 Aspirate and discard the top layer (plasma) solution to a remnant of 5 to 10 mm in height above the PBMCs interphase.
- 6.1.8 Carefully transfer the PBMCs layer above the porous barrier to a new 15 mL tube.
- 6.1.9 Wash the PBMCs with 10 mL of WM.
- 6.1.10 Centrifuge at 300 x g and 4°C for 10 min, then aspirate and discard the supernatant.
- 6.1.11 Re-suspend the cell pellet with 1 mL WM and transfer the cell suspension to microcentrifuge tube.
- 6.1.12 Centrifuge microcentrifuge tube at 300 x g and 4°C for 10 min, then aspirate and discard the supernatant.
- 6.1.13 For cell enumeration, re-suspend the cell pellet with 105 µL of WM and proceed to the protocol of “Cell Capture”.



Please click [Leucosep Preparation](#) for Abvideo™ instruction of operation.

Capture Antibody Immobilization

- 7.1 Wash the CytoChipNano with 200 μ L of ethanol followed by 200 μ L of 1X PBS twice. Make sure no bubble exists in the microfluidic channel.
- 7.2 Prepare Capture Antibody Working Solution by adding 50 μ L of Anti-EpCAM capturing antibody (Biotin) to 50 μ L of 1X PBS.
- 7.3 Pipette 100 μ L of Capture Antibody Working Solution into the chip and incubate the chip at RT for 1 hour in humidity box. Make sure the inlet and outlet of the chip are consistently covered with working solution throughout incubation.
- 7.4 Wash the CytoChipNano with 200 μ L of 1X PBS three times to remove the residual Capture Antibody Working Solution.
- 7.5 Prepare CytoQuest™ SuperBlocker Working Solution by adding 10 μ L of SuperBlocker to 90 μ L of 1X PBS.
- 7.6 Pipette 100 μ L of CytoQuest™ SuperBlocker Working Solution into the CytoChipNano and incubate the CytoChipNano at RT for 1 hour in humidity box. Make sure the inlet and outlet of the chip are consistently covered with working solution during incubation.
- 7.7 Wash the CytoChipNano with 200 μ L of 1X PBS three times to remove the residual CytoQuest™ Super Blocker Working Solution.
- 7.8 The CytoChipNano is now coated with capture antibody and is ready for use.



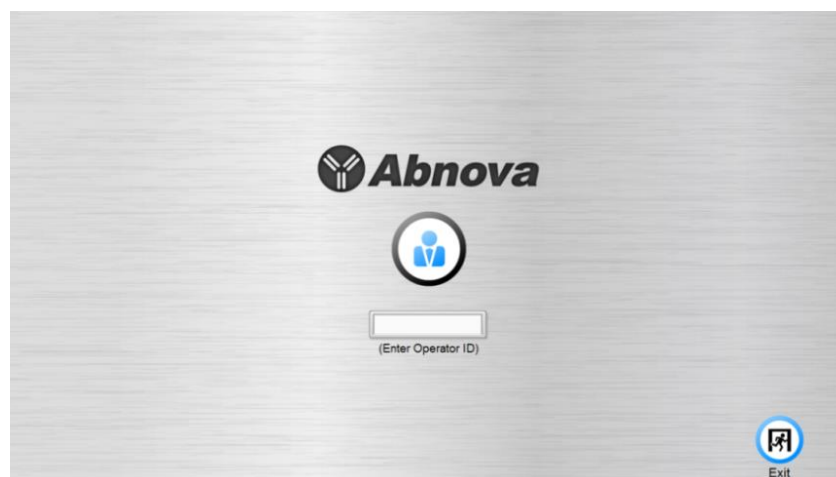
Please click [Capture Antibody Immobilization](#) for Abvideo™ instruction of operation.

8

Cell Capture

8.1 Parameters and Settings

- 8.1.1 Turn on the power switches of the liquid handler and thermal control of CytoQuest™ CR system.
- 8.1.2 Turn on the computer, and press the “S13001SP01-CytoQuest CR_v2.9” icon.



- 8.1.3 Enter or scan in “Operator ID”.



8.1.4 Press the “Custom Methods” icon.

8.1.5 Enter or scan in Barcode Contents ID for materials.



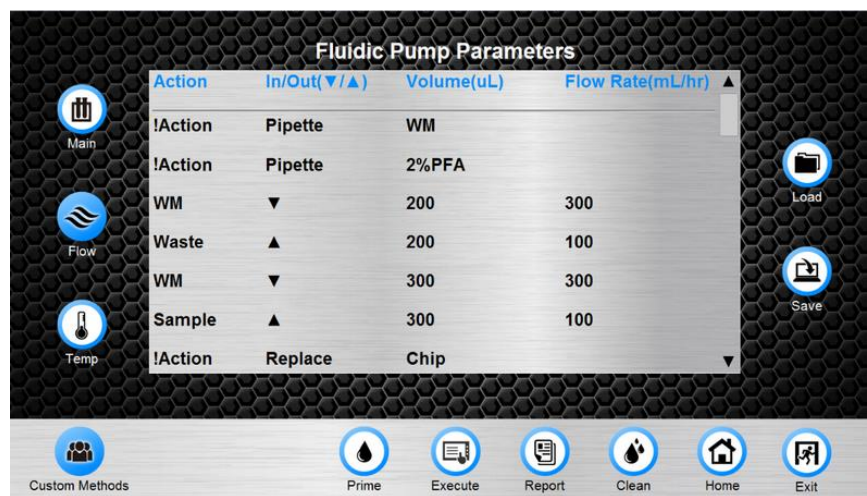
Material Parameters

Barcode Contents ID

Sample	Capturing Antibody	Antibody Dilution Buffer
CytoChipNano	Detecting Antibody 1	SuperBlocker
SCx™ Spiral Chamber	Detecting Antibody 2	Other

Navigation icons: Main, Flow, Temp, Custom Methods, Prime, Execute, Report, Clean, Home, Exit, Load, Save.

8.1.6 Press the “Flow” icon on the left side of the screen.

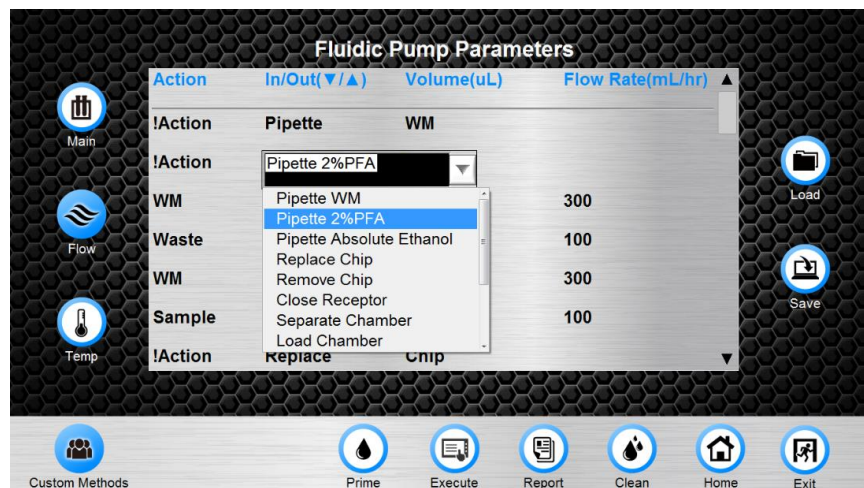


Fluidic Pump Parameters

Action	In/Out(▼/▲)	Volume(uL)	Flow Rate(mL/hr)
!Action	Pipette	WM	
!Action	Pipette	2%PFA	
WM	▼	200	300
Waste	▲	200	100
WM	▼	300	300
Sample	▲	300	100
!Action	Replace	Chip	

Navigation icons: Main, Flow, Temp, Custom Methods, Prime, Execute, Report, Clean, Home, Exit, Load, Save.

8.1.7 Setup the type of the fixation buffer in the Fluidic Pump Parameters.



- For Cell Immunostaining, 2% PFA is recommended and optimized for CytoQuest™ CR.
- For Cell Immunostaining plus Cell FISH, absolute EtOH is recommended and optimized for CytoQuest™ CR.
- For Cell Immunostaining plus Single Cell Micromanipulation, 2% PFA is recommended and optimized for CytoQuest™ CR.

8.1.8 The default temperature of the TCx™ Thermal Control is set at 22°C. Contact an Abnova Technical Representative if you would like to alter the default setting.

8.2 System Priming

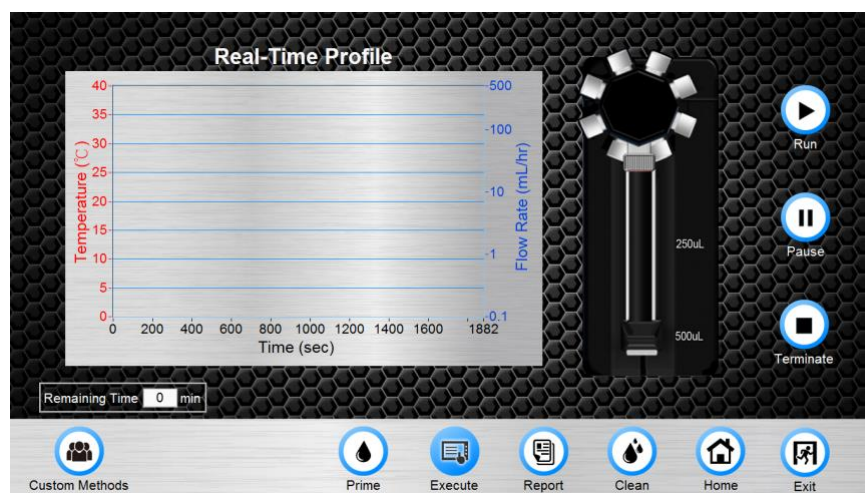
- 8.2.1 Press the “Prime” icon on the bottom tool bar to open the “Prime” page.
- 8.2.2 Place the Clean Chip in the Chip Receptor. Close the Chip Receptor. Insert the SCx™ Spiral Chamber into the Chamber Port. Then Insert the “Sample line” tubing into the SCx™ Spiral Chamber.



- 8.2.3 Press the “Run” icon to start the priming, and wait until this process is done.
- 8.2.4 Please proceed to the 8.3.1.

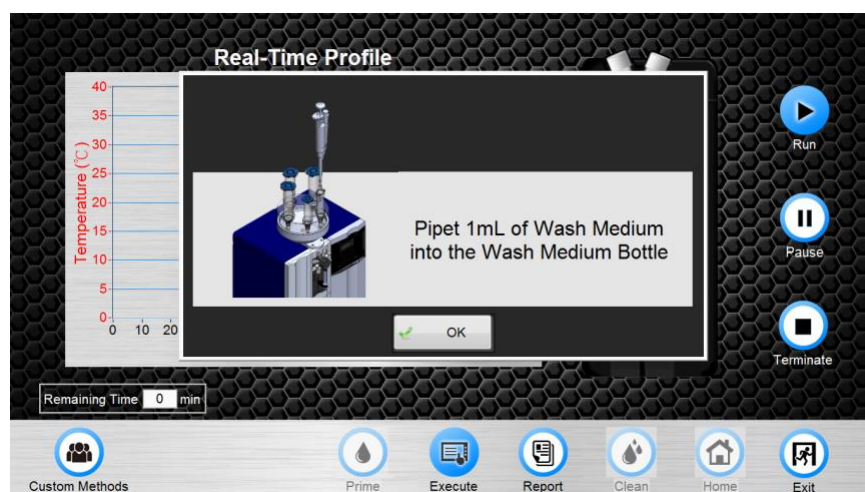
8.3 Buffer Filling

8.3.1 Press the “Execute” icon on the bottom tool bar to open the “Execute” page.

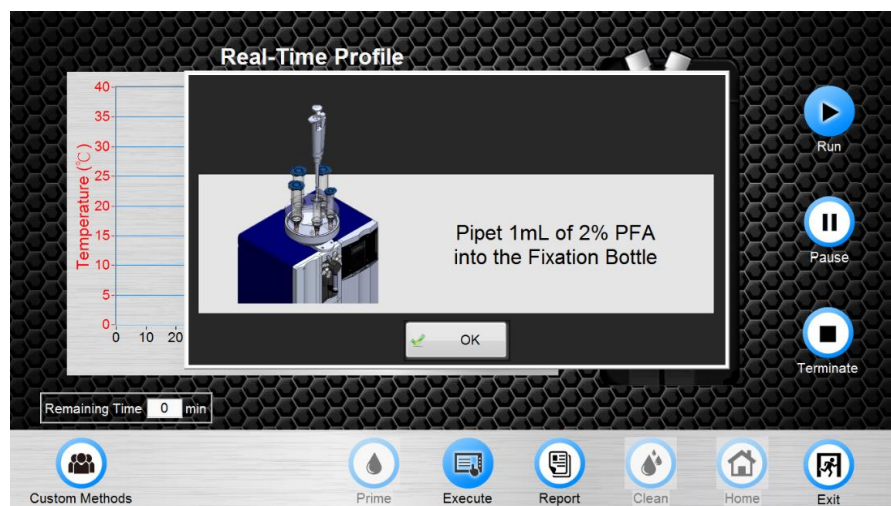


8.3.2 Press the “Run” icon to start the capture process.

8.3.3 Follow the instruction in popup menu: “Pipette 1 mL of Wash Medium into the Wash Medium Bottle” and press the “OK” icon.



- 8.3.4 Please follow the instruction in popup menu. Ex. “Pipette 1 mL of 2% PFA into the Fixation Bottle” and press the “OK” icon. The instruction varies with the fixation buffer setting in 8.1.7.

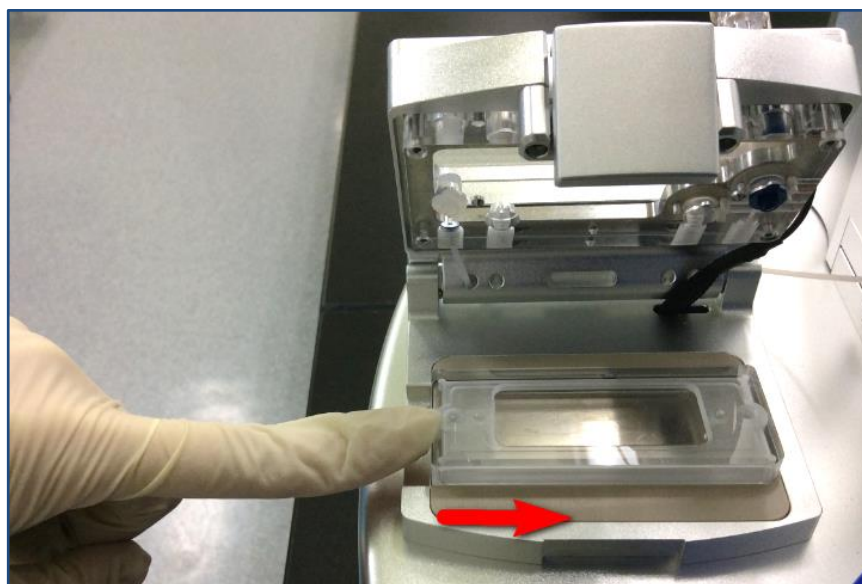


- 8.3.5 The Syringe Pump withdraws 200 μ L of Wash Medium. (300.0 mL/hour) from the Wash Medium Bottle.
- 8.3.6 The Syringe Pump discharges 200 μ L of Wash Medium into the waste bottle via Waste Line (100.0 mL/hour).
- 8.3.7 The Syringe Pump withdraws 300 μ L of Wash Medium (300.0 mL/hour) from the Wash Medium Bottle.
- 8.3.8 The Syringe Pump injects 300 μ L of Wash Medium into the SCx™ Spiral Chamber via Sample Line (100.0 mL/hour).

8.3.9 Follow the icon instruction in popup menu: “Open the Chip Receptor. Remove the Clean Chip and place capture antibody coated CytoChipNano”. Press the “OK” icon.



- Do not close the Chip Receptor.
- Place and push the CytoChipNano forward until it touches the opposing edge of the Chip Receptor.



- 8.3.10 The Syringe Pump withdraws 10 μ L of Wash Medium (300.0 mL/hour) from the Wash Medium Bottle.
- 8.3.11 The Syringe Pump injects 10 μ L of Wash Medium into the SCx™ Spiral Chamber via Sample Line (12.0 mL/hour).
- 8.3.12 Follow the instruction in popup menu: Close the Chip Receptor.” and press the “OK” icon.



8.4 Sample Loading and Capture

8.4.1 Follow the icon instruction in popup menu: “Disconnect the SCx™ Spiral Chamber from the Chamber Port. Separate the Connecting Base, hold the Coil Holder, and press the “OK” icon.



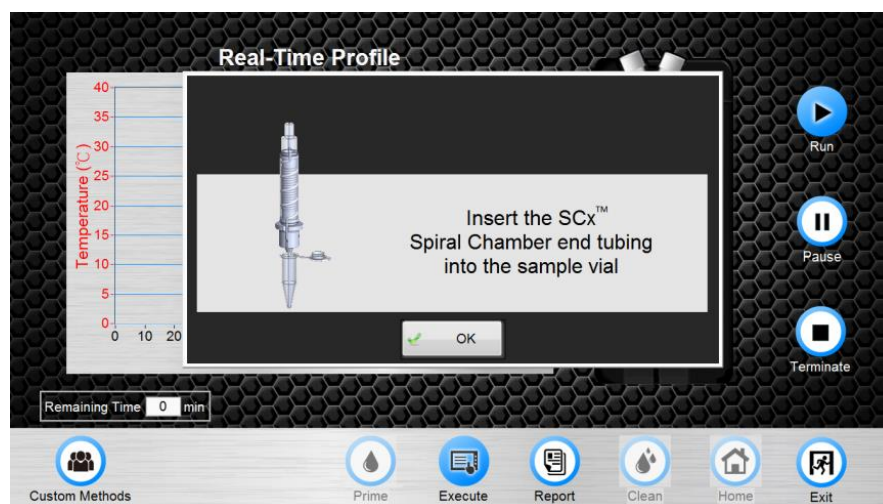
- Do not disconnect the SCx™ Spiral Chamber and Sample Line.
- Do not insert the SCx™ Spiral Chamber end tubing into the sample vial yet.



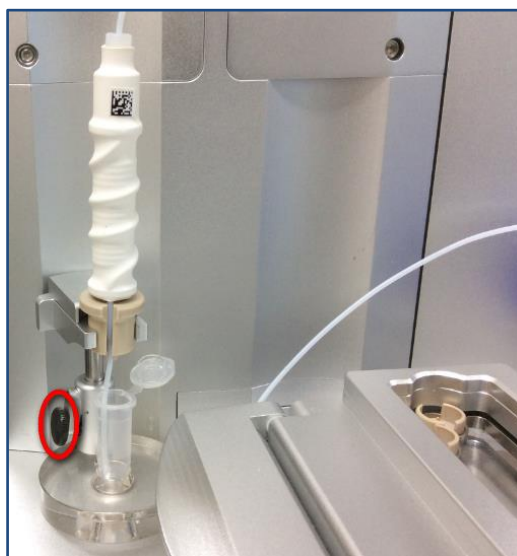
8.4.2 The Syringe Pump withdraws 20 μ L of Wash Medium from Wash Medium Bottle (300.0 mL/hour).

8.4.3 The Syringe Pump injects 20 μ L of Wash Medium into the SCx™ Spiral Chamber via Sample Line (12.0 mL/hour).

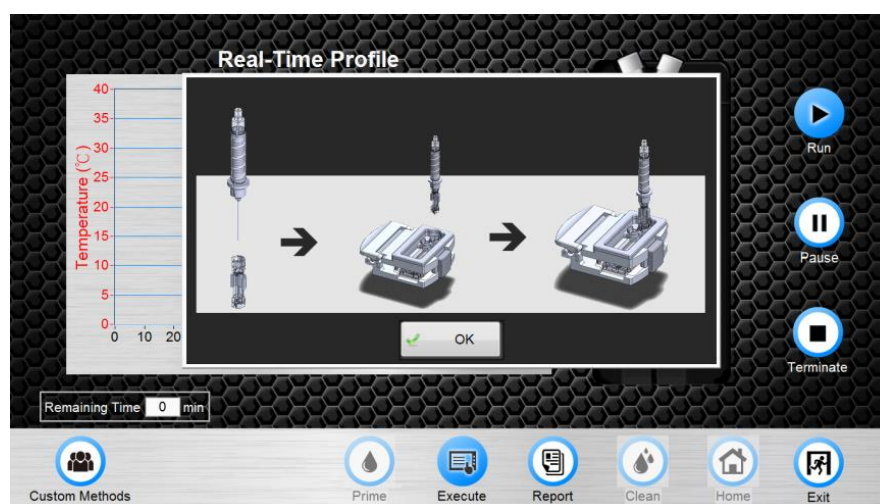
- 8.4.4 Follow the instruction in popup menu: “Insert the SCx™ Spiral Chamber end tubing into the sample vial”, and press the “OK” icon.



Place the SCx™ Spiral Chamber and the sample vial on Spiral Chamber Holder as shown below. Adjust height of stand via the adjustment screw indicated in the red circle in order to ensure the end of tubing reach the bottom of the sample vial.



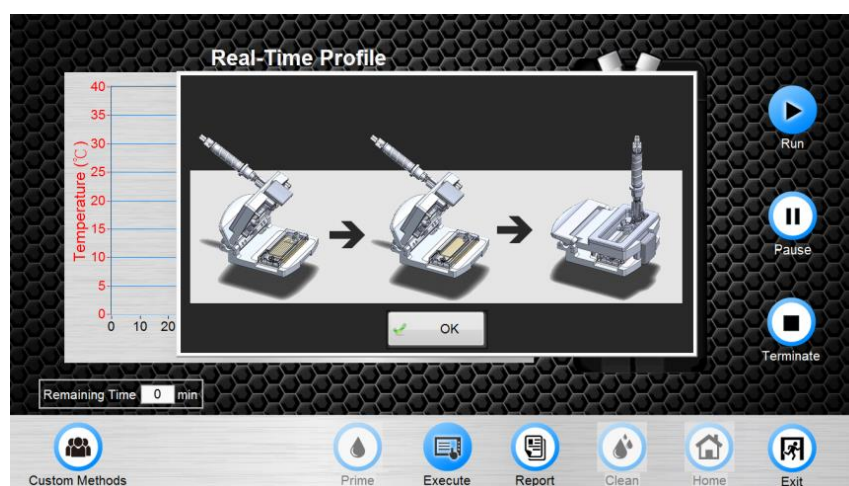
- 8.4.5 The Syringe Pump withdraws 100 μ L of sample (12.0 mL/hour).
- 8.4.6 The Syringe Pump discharges 100 μ L of Wash Medium into the waste bottle via Waste Line (100.0 mL/hour).
- 8.4.7 Follow the icon instruction in popup menu: “Assemble the SCx™ Spiral Chamber and connect to the Chamber Port gently” and press the “OK” icon.



- 8.4.8 The Syringe Pump withdraws 300 μ L of Wash Medium from the Wash Medium Bottle (300.0 mL/hour).
- 8.4.9 The Microvibrator in the SCx™ Spiral Chamber will turn on automatically.
- 8.4.10 The Syringe Pump injects 300 μ L of Wash Medium into the SCx™ Spiral Chamber via Sample Line (2.4 mL/hour).
- 8.4.11 The Syringe Pump withdraws 960 μ L of PBS (300.0 mL/hour).
- 8.4.12 The Syringe Pump injects 960 μ L of PBS into the SCx™ Spiral Chamber via Sample Line (4.8 mL/hour).
- 8.4.13 The Syringe Pump withdraws 300 μ L of PBS (300.0 mL/hour).
- 8.4.14 The Syringe Pump injects 300 μ L of PBS into the SCx™ Spiral Chamber via Sample Line (6.0 mL/hour).
- 8.4.15 The Microvibrator in the SCx™ Spiral Chamber will turn off automatically.

8.5 Cell Fixation

- 8.5.1 The Syringe Pump withdraws 200 μL of the fixation buffer from the Fixation Bottle (300.0 mL/hour).
- 8.5.2 The Syringe Pump discharges 200 μL of the fixation buffer into the waste bottle via Waste Line (100.0 mL/hour).
- 8.5.3 The Syringe Pump withdraws 400 μL of the fixation buffer from the Fixation Bottle (300.0 mL/hour).
- 8.5.4 The Syringe Pump injects 400 μL of the fixation buffer into the SCx™ Spiral Chamber via Sample Line (2% PFA: 4.8 mL/hour; absolute EtOH: 2.4 mL/hour).
- 8.5.5 Follow the icon instruction in popup menu: “Replace the CytoChipNano with Clean Chip” and press the “OK” icon.



- 8.5.6 Allow the CytoChipNano to sit for 15 minutes if 2% PFA was used as the fixation buffer.
Allow the CytoChipNano to sit for 25 minutes if absolute EtOH was used as the fixation buffer.
- 8.5.7 Cell Capture process is now finished. Please proceed to “Cell Immunostaining”.

8.6 Tubing Cleaning

- 8.6.1 The Syringe Pump withdraws 500 μ L of deionized water (300.0 mL/hour).
- 8.6.2 The Syringe Pump discharges 500 μ L of deionized water into the SCx™ Spiral Chamber via Sample Line (100.0 mL/hour).
- 8.6.3 The Syringe Pump withdraws 500 μ L of 70% EtOH (300.0 mL/hour).
- 8.6.4 The Syringe Pump discharges 500 μ L of 70% EtOH into the SCx™ Spiral Chamber via Sample Line (100.0 mL/hour)
- 8.6.5 The Syringe Pump withdraws 500 μ L of PBS (300.0 mL/hour).
- 8.6.6 The Syringe Pump discharges 500 μ L of PBS into the SCx™ Spiral Chamber via Sample Line (100.0 mL/hour).



Please click [Cell Capture](#) for Abvideo™ instruction of operation.

Cell Immunostaining

- 9.1 Pipette 200 μ L of 1X PBS into the CytoChipNano three times to remove the residual fixation buffer.
- 9.2 Pipette 200 μ L of 1X ADB/0.2% Triton X-100 into the CytoChipNano for 20 minutes.
- 9.3 Pipette 200 μ L of 1X PBS into the CytoChipNano three times to remove 1X ADB/0.2% Triton X-100.
- 9.4 Prepare the detecting antibody cocktail by adding 6.25 μ L of Anti-PanCK detecting antibody (FITC) and adding 1.25 μ L of Anti-CD45 detecting antibody (PE) to 250 μ L of 1X ADB.
- 9.5 Pipette 250 μ L of detecting antibody cocktail into the CytoChipNano and incubate it away from light at room temperature for 1 hour in humidity box.
- 9.6 Pipette 200 μ L of 1X PBS into the CytoChipNano three times to remove the residual detecting antibody cocktail.
- 9.7 Pipette 200 μ L of 300 nM DAPI solution into the CytoChipNano and wait for 10 minutes.
- 9.8 Pipette 200 μ L of 1X PBS into the CytoChipNano to remove the residual DAPI solution and incubate it away from light at room temperature.
- 9.9 Cell Immunostaining in the CytoChipNano is now ready for “Cell FISH” or microscope observation. Please refer to recommended filter sets listed for each fluorochrome below. For single cell micromanipulation, please refer to CytoQuest™ Single Cell Picking Kit (http://www.abnova.com/protocol_pdf/KA4578.pdf).

Fluorochrome	EX(nm)	EM(nm)	Recommended Filter Set
APC	650	660	Cy5-4040C, SemRock
DAPI	359	461	49000, Chroma
FITC	490	525	39002, Chroma
R-phycoerythrin (PE)	565	578	49010, Chroma
TRITC	555	580	31002, Chroma



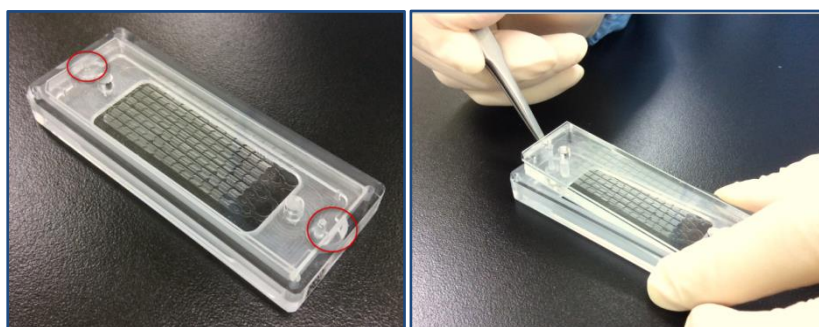
Please click [Cell Immunostaining](#) for Abvideo™ instruction of operation.

10

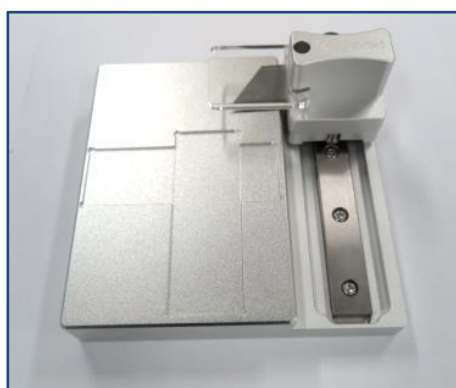
Cell FISH

Slide preparation

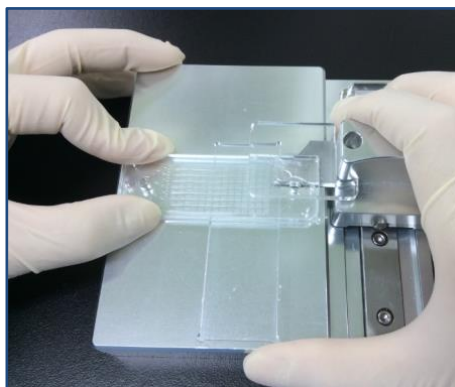
- 10.1. Prepare 200 μ L of air into the CytoChipNano twice to remove 1X PBS.
- 10.2. Flip the CytoChipNano so the bottom side faces up. There are 2 semi-circular notches. Gently insert the tip of the tweezer into the notch then lift the CytoChipNano up from the Bento Cover below.



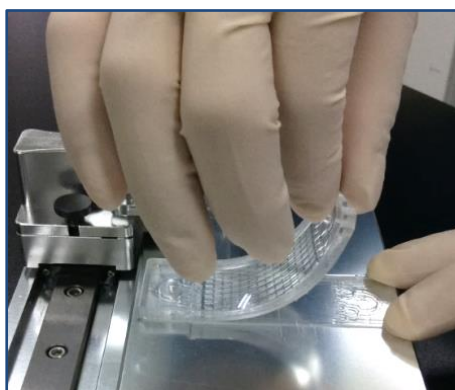
- 10.3. Setup the Chip Opener with blade on the top position.



- 10.4. Place the CytoChipNano right-side up on the indent. Hold down the ends of the CytoChipNano and cut the four sides of the CytoChipNano with sliding blade. Do not press the middle of the CytoChipNano.



10.5. Gently detach the micromixer carefully from the slide.



Slide pre-treatment

- 10.6 Immerse the slide in 2X SSC at room temperature for 15 min.
- 10.7 Immerse the slide in 2X SSC at 37°C for 30 min.
- 10.8 Dehydrate the slide by immersing the slide in a series of EtOH solutions (70%, 85% and 100%; 2min each) at RT.
- 10.9 Air-dry the slide.

Probe preparation

- 10.10 Briefly vortex and centrifuge the probe mix.
- 10.11 Prepare the probe mix as per manufacturer's instructions.
- 10.12 Briefly vortex and centrifuge the tube.
- 10.13 Denature the probe at 75°C for 5 min.
- 10.14 Briefly centrifuge the tube.

- 10.15 Warm up the slide on a hotplate at 45°C for 5 min before applying the probe.
- 10.16 Apply 10 µL of the probe mix to the slide and immediately apply the coverslip on the slide.
- 10.17 Seal the coverslip with rubber cement.

Co-denaturation

- 10.18 Warm the slide on a hotplate at 80°C for 2 min for denaturation of target and probe DNA.
- 10.19 Hybridize at 37°C for 36-48 hr.

Washing the slide

- 10.20 Carefully remove the rubber cement and coverslip by securing the coverslip between your index finger and thumb.
- 10.21 Slowly peel off the rubber cement with forceps.
- 10.22 Carefully slip the coverslip off the slide without lifting it.
- 10.23 Immediately immerse the slide in 2X SSC/0.3% NP-40 at 72°C for 2 min.
- 10.24 Immerse the slide in 2X SSC at RT for 5 min.
- 10.25 Air dry the slide in the dark and keep the slide in an upright position.

Counter Stain and Mounting

- 10.26 Apply 20 µL of DAPI counterstain to the target area and apply coverslip. Place the slide at RT for 15 min.
- 10.27 The slide is now ready for examination under fluorescence microscope.



Please click [Cell FISH](#) for Abvideo™ instruction of operation.

11

System Maintenance

11.1 General Maintenance

For reliable operation, keep the instrument free from dust and liquid spills. We recommend cleaning the instrument periodically. A soft cloth dampened in mild detergent is sufficient. Please operate the instrument at least once a year. If any surfaces have been contaminated with bio-hazardous material, a sterilizing solution must be used.

Buffer bottles on top of the instrument are filled with DI water (yellow bottle tubing), 70% ethanol (green bottle tubing), and 1X PBS (blue bottle tubing). The white instrument tubing is washed by these buffers. To avoid contamination, wash buffer bottles and replace the content with fresh buffers once a week. For conduct maintenance, users should always perform “System Cleaning” after operation.

11.2 System Cleaning

11.2.1 Press the “Clean” icon on the bottom tool bar to open the “Clean” Page.

11.2.2 Place the Clean Chip into the Chip Receptor.



11.2.3 Press the “Run” icon to start the cleaning process, and wait until it is done.

11.2.4 Turn off the power switches of the liquid handler and the thermal control of CytoQuest™ CR system.



Please click System Maintenance for Abvideo™ instruction of operation.

12

System Specifications

Hardware Specifications

General Specifications	
Dimensions:	565.06mm(h) x 340.8mm(d) x 271.8mm(w)
Net Weight:	18 kg
Power:	Liquid Handler Unit: 100-240VAC, 50/60Hz, 1.6A Thermo Control Unit: 85-250VAC, 50/60Hz, 4.0A
Replace Fuse:	Liquid Handler Unit: 1.6A(Peak)/250V Thermo Control Unit: 4A (Peak)/250V
Operating Temperature:	60°F to 104°F (15°C to 40°C)
Programable Thermo Control Profile:	4-40°C, Variable Time Span

Environmental Specifications	
Storage Temperature:	-5°F to 158°F (-20°C to 70°C)
Operation Altitude:	Up to 2000m
Operation Environment:	For Indoor Use
Operating Humidity:	20% to 90% Relative Humidity, Non-Condensing
Storage Humidity:	20% to 95% Relative Humidity, Non-Condensing

Software Specifications

General Specifications	
Scanner Function:	Scanning Capability to Read QR Code/Barcode
Status Display:	Progress and Fluid Handling
System Clean:	Prime and Clean
Report Generation:	Print and Export File
Necessary Driver:	LabVIEW Run-Time Engine
	2011 SP1-(Standard RTE)
	NI-VISA Run-Time Engine 5.4
	ATEN_32/64bit_Driver_v2.0.7.0_Binary

Touchscreen Laptop Specifications

General Specifications	
Operating System:	Windows 8/7/Vista (32-bit and 64-bit)
	Windows XP SP3 (32-bit)
	Windows Server 2003 R2 (32-bit)
	Windows Server 2008 R2 (64-bit)
Processor:	Pentium III/Celeron 866 MHz or Equivalent
Memory:	256MB Minimum (512MB Recommended)
Free Disk Space:	1GB
Interfaces/USB Ports:	USB 3.0 Port *1

Symbols

 Manufacturer	 Serial Number	 Electronic specification
 Date of manufacture	 Caution	 Consult instructions for use
 Protective Conductor	 WEEE	

 **Abnova (Taiwan) Corporation**
 3rd Fl., No.132, Xiyuan Rd.
 Zhongli District, Taoyuan City,
 Taiwan

Abnova (Taiwan) Corporation

9F, No. 108, Jhouzih St., Neihu, Taipei 114, Taiwan

Tel: + 886 2 8751 1888 Fax: + 886 2 6602 1218

